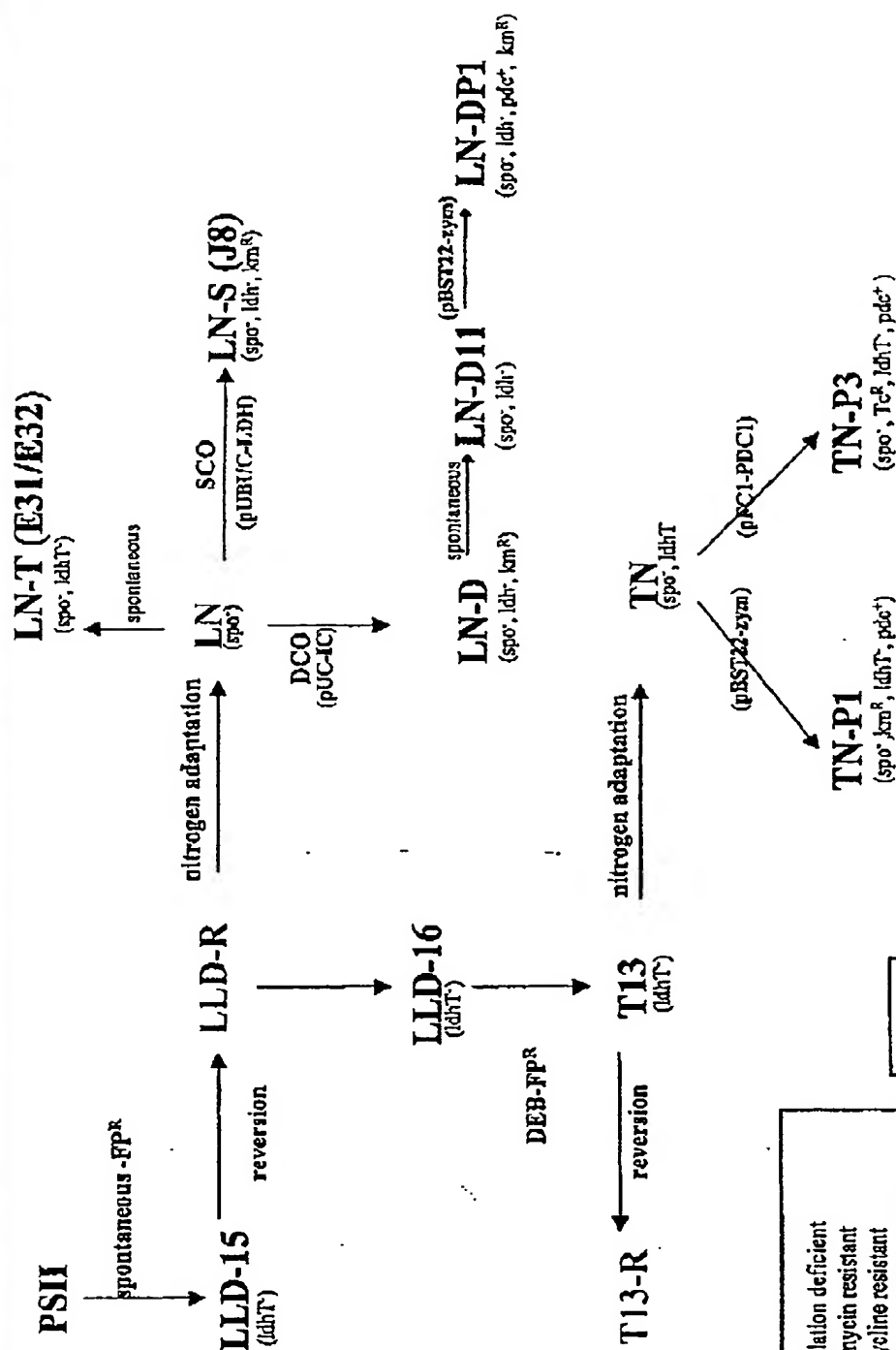


Figure 1

## Ethanol Strain Development



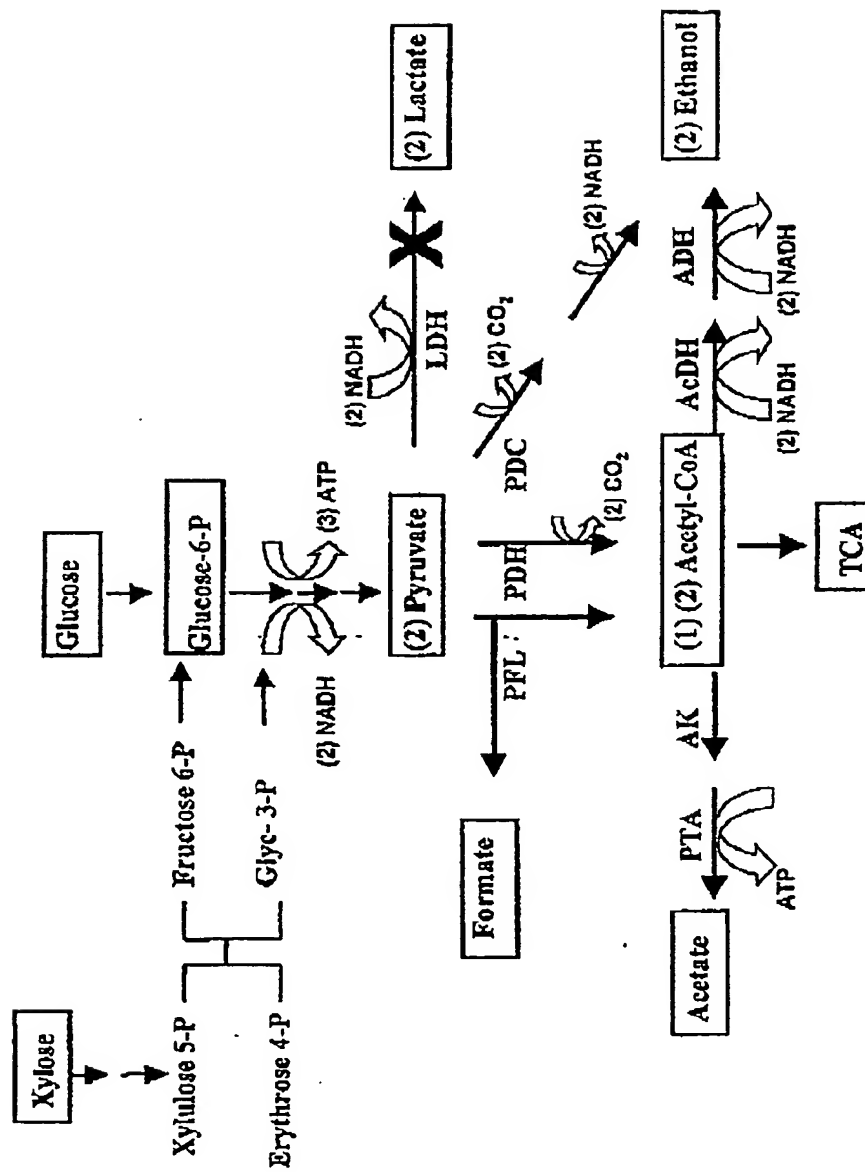
ethanol  
lactate

spo<sup>-</sup> sporulation deficient  
km<sup>R</sup> kanamycin resistant  
Tc<sup>R</sup> tetracycline resistant  
ldh<sup>-</sup> LDH recombination mutant  
ldhT<sup>-</sup> LDH transposon mutant  
pdc<sup>-</sup> pyruvate decarboxylase positive

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## Sugar Metabolism to Ethanol

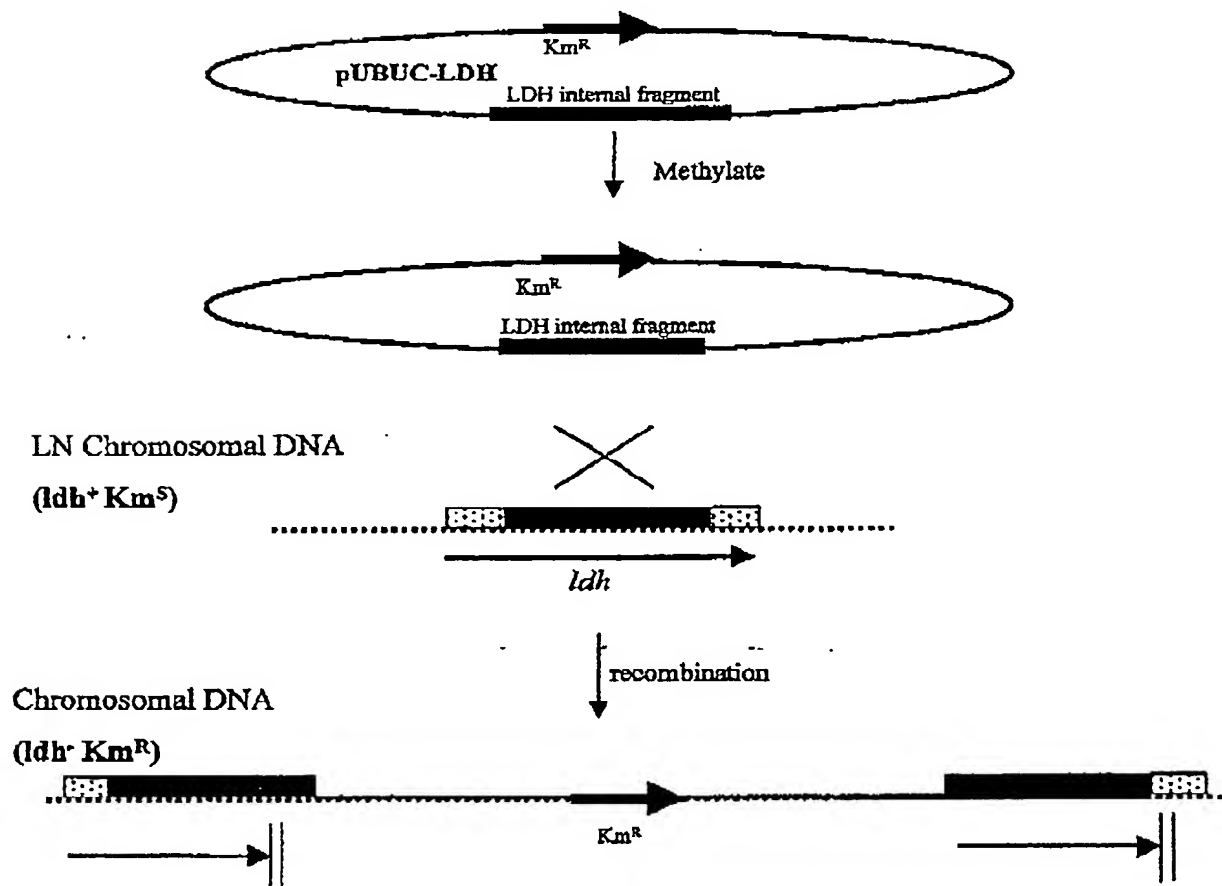
Figure 2



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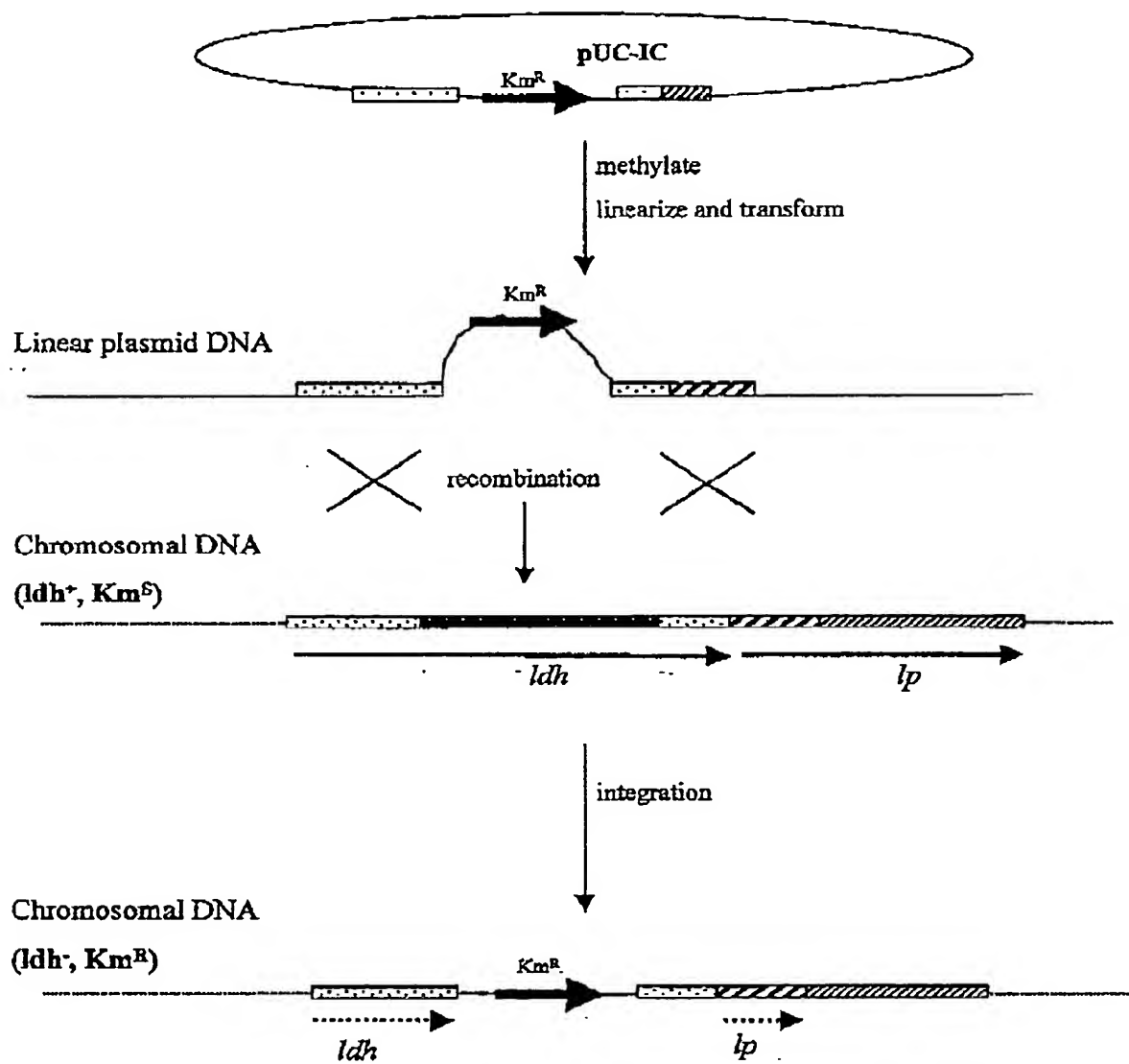
Figure 3

### LDH Gene Inactivation by Single-Crossover Recombination



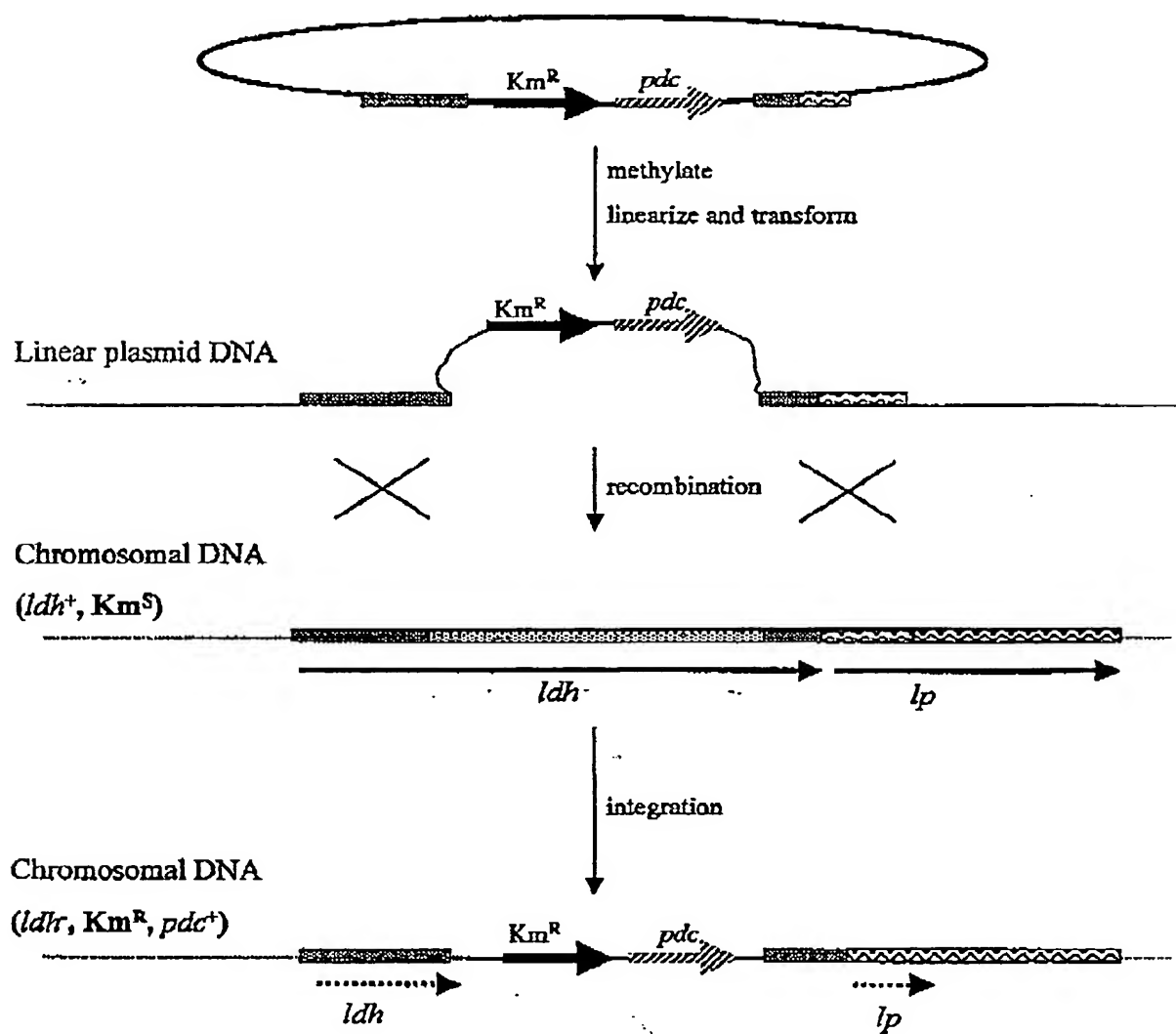
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Figure 4

**LDH Gene Inactivation by Double-Crossover Recombination**

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Figure 5

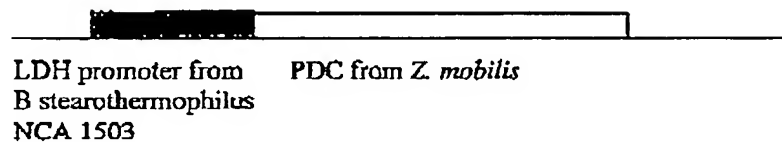
**LDH Gene Inactivation and Heterologous *PDC* Gene Expression**

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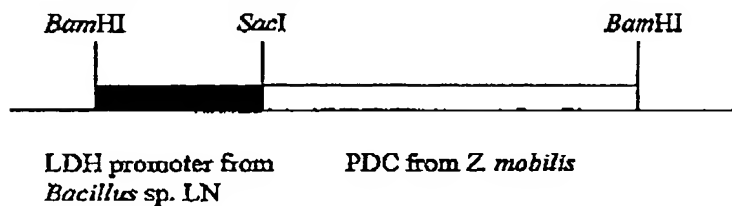
Figure 6

## Expression of PDC

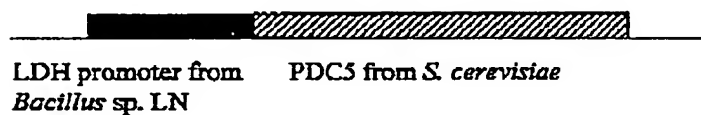
### Construct 1 (cloned in pBST22)



### Construct 2 (cloned in pFC1)



### Construct 3 (cloned in pFC1)



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Figure 7. LDH promoter sequence from *Bacillus* LN

AGGGCAATCTGAAAGGAAGGGAAAATTCCTTTCCGATTGTCCTTTTAGTTATTTTATGG - 60  
GGACTGAATATTATATAGGCATTACGGAAATGATAATGGCAGAGTTTTTTCATTTATTAG - 120  
ACTGCTTGATGTAATTGGATGTGATGATACAAAATAATGTTGTGTAAACAAAATGTTAA - 180  
CAAAAAGACAAATTTCATTCATAGTTGATACTTGATAAAGATTGTGAATAATGCACAA - 240  
TATATCAATGTATGAGCAGTTTCACAAATTCATTTTTTGGAAAGGATGACAGACAGCG'AT - 300

G

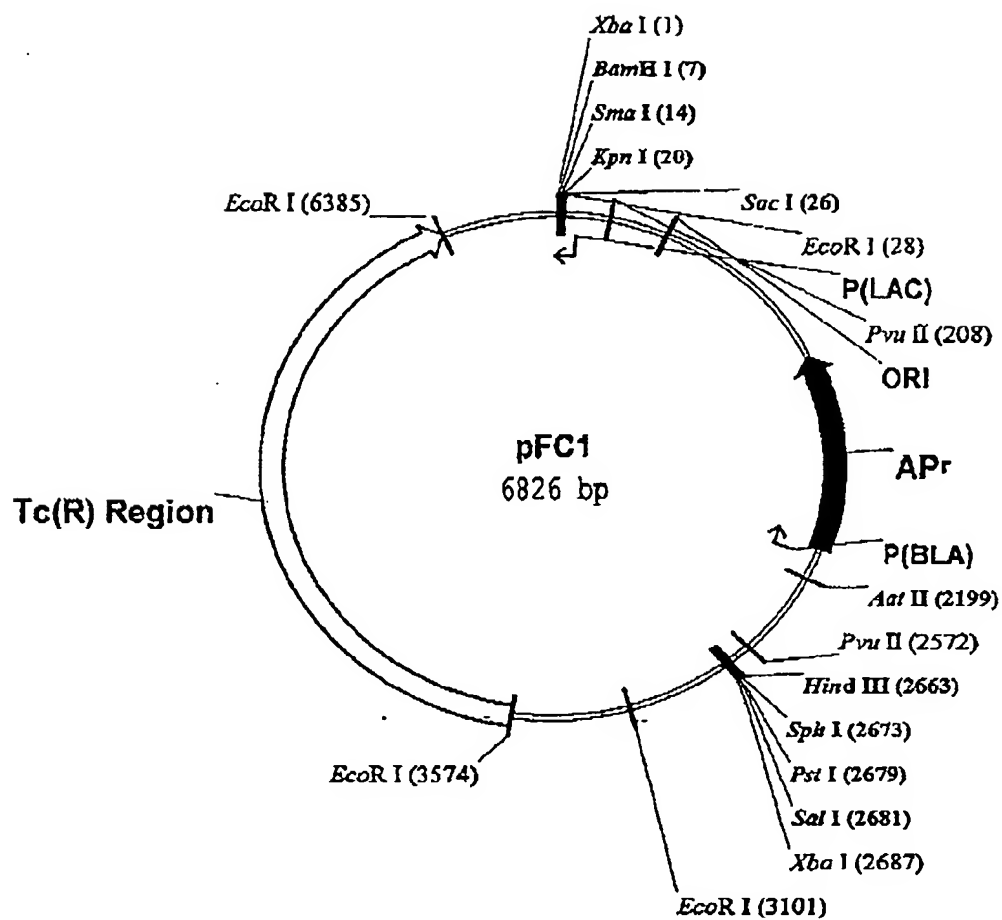
underlined: putative promoter sequences

**bold**: putative ribosome binding site

\*: start codon

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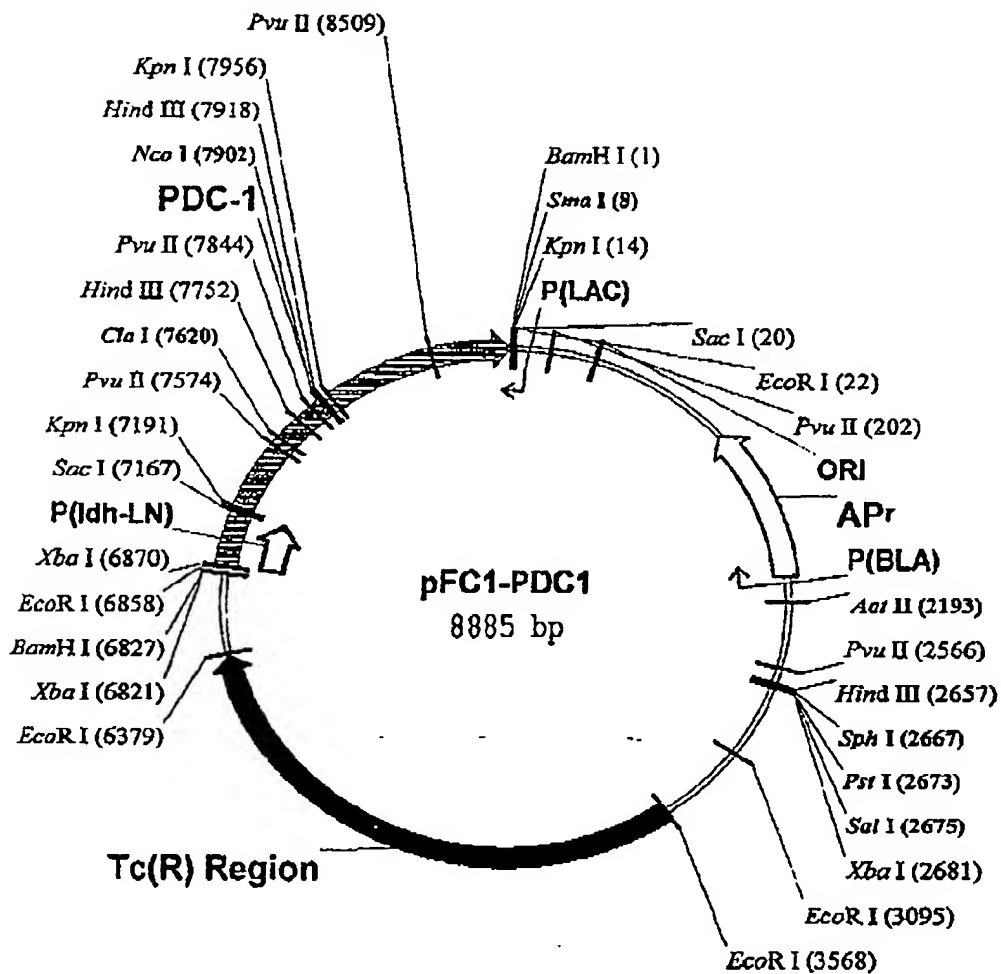
Figure 8





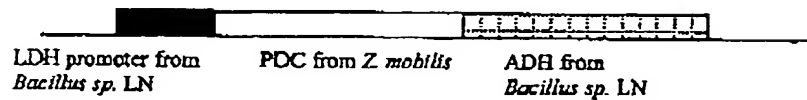
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Figure 9



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Figure 10

**Construction of an Artificial PDC operon****Construct 4****Construct 5**